

Subculture of 2 Overnight Culture of LT10 Pro Transformed into BL21 Competent Cells

Made with Benchling

Project: Awesome Possum

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MONDAY, 10/10

Purpose:

Subculture 5.0 mL overnight cultures into larger volume of fresh media in preparation for protein induction.

Materials:

- 2X 5.0 mL overnight cultures of LT10 Pro in BL21
 - Colony "3B" and Colony "2A"
- Micropipette
- Micropipette tips
- Cuvettes
- Implen P 300
- 1.0 L glass shake flask
- Terrific Broth
- Chloramphenicol
- Shaker/Incubator

Protocol:

The 2 overnight culture samples were retrieved from the shaker/incubator at 8:30 am. Both samples were very clear and no cloudyness was noted. A 1.0 mL sample was retrieved from each culture tube and stored in eppendorf tubes for optical density measurements. Next, 2 1.L shake flasks were filled with 300.0 mL of Terrific Broth and 300.0 μ L of chloramphenicol. After that, the 4.0 mL overnight samples were both delivered to the corresponding shake flasks. Then, the flasks were covered with tin foil, labeled, and placed inside a shaker/incubator at 37.0°C 250 RPM.

A nano-photometer blank was prepared the night before using 1.0 mL of Terrific Broth and 1.0 μ L of chloramphenicol. The Implen P 300 was blanked at OD600 with the prepared blank. Next the initial overnight samples were measured at OD600. The results were noted in Table 1.

Using the same prepared blank, the 2 cultures were measured at OD600 every hour until they reached an OD600 value for which induction with IPTG was required. The colony 3B culture was discarded after the 11:00 AM OD600 check. The culture failed to progress (still completely clear) and was considered of nil significance. The hourly OD600 measurements were noted in Table 2.

Table 1: OD600 of Overnight Cultures

	A	B
1	<u>Colony #</u>	<u>OD600</u>
2	3B	0.01
3	2A	0.093

Table 2: OD600 Hourly Measurements of Subcultured LT10 Promoter in BL21 Cells

	A	B	C
1	<u>Time</u>	<u>OD600 Colony</u> <u>2A</u>	<u>OD600 Colony</u> <u>3B</u>
2	10:18 AM	0.016	0.014
3	11:15 AM	0.022	0.019
4	1:17 PM	0.205	N/A
5	2:13 PM	0.727	N/A

At 2:13 PM culture colony 2A reached an inducible OD600 of 0.727. A 1.0 mL sample was retrieved inside 1.5 mL eppendorf tube labeled Fraction 1 "non-induced." The culture was removed from the shaker/incubator and 3.0 mL of 100 mM IPTG was added to the flask at 2:37 PM. After that, the flask was returned to the shaker/incubator at 37.0°C 250 RPM. The culture was allowed to grow until 5:30 PM. Then, the culture flask was placed inside a 4.0°C refrigerator overnight.

TUESDAY, 10/11

The chilled culture was divided evenly into 2 large centrifuge tubes, which were balanced in preparation. The tubes were later centrifuged at 9,500 RPM for 15 minutes. Next, the supernatant was decanted into a screw-top bottle and the pellet was scraped into a 50.0 mL screw top falcon tube. The supernatant was stored in a 4.0°C refrigerator, while the pellet was stored inside a -20.0°C freezer.